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A COMPREHENSIVE METHOD FOR THE DETERMINATION OF TOTAL POTATO GLYCOALKALOIDS[1]

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ABSTRACT

A method has been developed for the quantitative analysis of total glycoalkaloids (TGA) in potato tubers. The method consists of TGA extraction by suitable solvent mixtures followed by hydrolysis of the glycosides and extraction of the aglycones. The aglycones are then quantitated by nonaqueous titration. The advantages of this method over those previously described are the inclusion of glycoalkaloids that are not measured by other methods, and the simplicity, safety, and rapid nature of the procedure. This method has been applied to the TGA analysis of potato tubers subjected to a variety of storage and treatment conditions.

INTRODUCTION

The relationship of the glycoalkaloids to potato quality, and resistance to disease and insect infestation has been studied (1, 5, 8). Recognition of the potential toxicity of the glycoalkaloids in new potato varieties bred specifically for disease resistance, has increased the need for a simple, rapid analytical procedure for measuring total glycoalkaloid (TGA[3]) content.

Several methods for the determination of glycoalkaloids are in the literature (3, 4, 7), which has recently been extensively reviewed (9).

Currently, the methods used most rely on two basic steps: ammonia precipitation of glycoalkaloids and their spectrophotometric quantitation (3, 12).

While precipitation will isolate glycoalkaloids such as solanine, chaconine, and demissine (which are thought to constitute the greater portion of tuber TGA), it does not include the leptines and other compounds of similar structure or solubility characteristics. The toxic properties of these soluble glycoalkaloids have yet to be determined.

Spectrophotometric determination of the precipitated glycosides is normally carried out using such reagent combinations as sulphuric acid-formaldehyde, phosphoric acid-paraformaldehyde, and more commonly, antimony trichloride in hydrochloric acid (3, 7, 12). The corrosive and toxic nature of these reagents requires extreme caution in their routine use. In addition they require the presence of a double bond in the aglycone for any significant color formation. Therefore, demissine, which may be present in ammonia precipitates, being saturated, would not be measured. These methods, therefore, are essentially limited to the measurement of solanine and chaconine. Further, these reagents will react with steroids if present as contaminants in the extracts.

We are reporting a method of analysis that overcomes the inadequacies of these current photometric methods; one which measures all of the glycoalkaloids irrespective of their solubility or their degree of saturation, and does not include non-nitrogenous steroids. In addition this method

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[3]This term will apply to any value that ultimately can be converted to total glycoalkaloids.

tilizes only common chemicals and inexpensive and simple equipment. The analysis may be completed in about four hours, three of which are evoted to settling and evaporation of solutions, during which time little ttention of the analyst is required. Multiple analyses can be carried out imultaneously with a minimum of effort.

This analytical method for TGA consists of three major steps: otato sampling and crude glycoside extraction; the quantitative isolation f these compounds; and finally the measurement of TGA. Similar ıethods have been used to determine solasodine in *Soltnum lactiniatum* ut with a slower and more complicated method of TGA isolation and nal glycoside measurement (2).

No effort was made to evaluate the many methods of potato sampling ›r analysis, therefore we do not conclude that the method of initial lycoside extraction which was incorporated into this procedure because f speed, handling facility, and modest equipment requirements is superior › other available methods.

Materials and Methods

Sampling: To obtain a representative aliquot, a 5 lb sample of potato ıbers is scrubbed, and ranked by size in a row from largest to smallest. ‹very other tuber is selected from which paired opposite eighths (wedges) re cut longitudinally. The total weight of these wedges is normally ca.)0 g. These pieces are immediately chopped fine (not pureed) either y hand or in a blendor without addition of liquid and from this mixture random 20.0 g sample of fresh tuber material is taken for analysis. This ıould be completed in about 5 min before enzymatic action can take lace to any extent.

Coarse extraction of TGA: A modification of the bisolvent method ‹ported by Wang (11) is used. The 20 g sample of potato is ground in Waring[4] blendor (500 ml jar) with 100 ml of 2:1 methanol-chloroform ıixture for 5 min. The mixture is filtered through a coarse fritted glass ınnel directly into a 500 ml separatory funnel fitted with a Teflon® stop›ck, pushing liquid through the filter with slight nitrogen or air pressure. 'uber residue is returned to the blendor and reextracted for 3 min with) ml of 2:1 methanol-chloroform mixture. Refilter, rinsing blendor and ‹sidue with 30 ml of the solvent mixture (for a total of 200 ml). Expel quid from the filter cake with nitrogen pressure. One hundred ml of .8% Na_2SO_4 solution is added to the filtrate in the separatory funnel 'hich is then shaken vigorously to separate the bisolvent system. Clearing f the layers occurs in ca. 2 hours but scheduling afternoon extractions 'ill permit complete separation to take place overnight. The lower (chloro›rm) layer is discarded and the methanol layer filtered through What›an No. 1 paper and made to 250 ml with methanol.

Quantitative isolation of TGA: Evaporate duplicate aliquots (25 ml ı most cases — 50 ml if TGA is expected to be low) of the aqueous ıethanol layer to dryness. Evaporate in a warm water bath under a jet f nitrogen. Redissolve in 15-20 ml of absolute methanol, and filter through Vhatman No. 1 paper into 50 ml flasks. This will effectively separate most of the sodium sulphate, which should be washed several times more on the filter with a few ml of absolute methanol. Evaporate filtrate to dryness, redissolve in 15 ml of 2N H_2SO_4 and hydrolyze on steam bath for 2 hours after fitting the flask with an air condenser. The hydrolysate is then made basic with 7-8 ml of 4N NaOH. There is usually a color change as the solution shifts to the basic side but the pH should be checked with indicator paper. Transfer the brown aqueous solution to a separatory funnel and extract with three 15 ml portions of benzene. Emulsion at the interface can be broken by vigorous agitation. If separation is incomplete, centrifugation after the third extraction and removal of the benzene by syringe will suffice. Wash the combined benzene extracts twice with water to remove any residual alkali. The final benzene solution should not contain visible water droplets, which could introduce error in the final TGA determination, however, trace amounts of water need not be removed with drying agents as concentrating the benzene solution provides sufficient drying.

Determination of TGA: The benzene solution containing the isolated aglycones is evaporated to dryness in a warm water bath with a jet of N_2, (or air) being careful not to overheat. Redissolve the residue in 5 ml absolute methanol. This solution is then titrated with a solution of 0.067% (10^{-3} M) bromphenol blue (3′, 3″, 5′, 5″ Tetrabromosulphonephthalein) and 10% phenol (analytical grade - Malloncrodt) in absolute methanol, the color passing from blue through blue-green to a yellow end point, the original reagent color. A 10 ml semimicro buret graduated in 0.02 ml is used. The nonaqueous titration measures the basic nitrogen group common to all of the aglycones. Blank titrations of the methanol should be subtracted from the total. Standardize the titration solution against a known concentration of solanine in methyl alcohol. Tomatine, which is more readily available, may be used, athough purity should be known; 1.00 mg tomatine is equivalent to 0.84 mg solanine or chaconine. In the concentration used, 1.00 ml titrant is equivalent to 0.867 mg solanine.

Calculations: With 20.0 gm potato tissue represented by 250 ml extract and a bromphenol blue solution standardized so that 1.0 ml≃0.87 mg of solanine and a blank titration of 0.08 ml for 5 ml of absolute methanol.

If a 50 ml aliquot of extract after hydrolysis and extraction gave a titer of 0.88 ml — blank of 0.08 ml = net titer of 0.80 ml, then: $0.80 \times .87 = .696 \text{ mg} \times \frac{250}{50} = 2.784$ mg/20 g fr. wt., therefore, $2.784 \times 5 = 13.92$ mg/100 g fr. wt. of potato.

Results and Discussion

The stoichiometric nature of the titration is shown by the calibration line in Fig. 1. Purified α-solanine (6) was dissolved in methanol and titrated as described.

The endpoint of the titration is easily recognized and easily reproduced. Measured replicate samples of solanine in methyl alcohol, titrated with the nonaqueous bromphenol blue reagent could be easily reproduced to the second decimal place. To study reproducibility of the entire procedure, four replicate analyses of tuber material from the same composite

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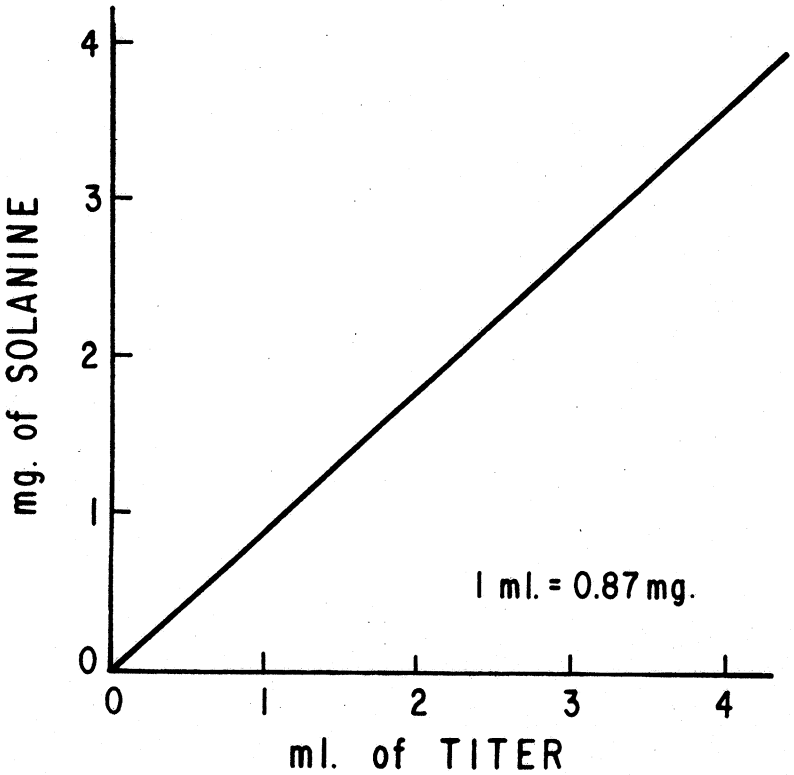

FIG. 1.—Titration of solanine with bromphenol blue.

potato preparation were carried separately through the entire procedure. The results are contained in Table 1.

Recovery experiments were carried out in which solanine was added to tuber extracts, which were then carried through the entire procedure. Table 2 shows the results. Recoveries were at least 94% over a range equivalent to ½ to 10 times the TGA originally present in the tuber.

When lower concentrations, ca. 0.1 to 0.2 mg of solanine are encountered where the titer is small and may cause the end point to be misinterpreted, the bromphenol blue solution may be diluted at least 5 to 1 with absolute methanol and the endpoint will be quite reproducible and in direct proportion to the dilution. More satisfactory results were obtained, however, when the amount of tuber extract taken for analysis contained ca. 0.5 mg of TGA or higher.

It was assumed that demissine and similar saturated compounds would be more difficult to hydrolyze. Quantitative recoveries of tomatine, the only available saturated glycoside were obtained under the given conditions for hydrolysis, at the 1.0 mg level.

To insure that potato amino acids were not contributing nitrogen tc be measured in the final titration, a mixture of amino acids known to be present in potatoes (10), was carried through the hydrolysis extraction,

TABLE 1.—*Precision of analytical procedure.*

Tuber sample no.[1]	mg TGA/100 g fr. wt.
1-A	9.52
1-B	8.40
1-C	9.68
1-D	8.40
Mean	9.00, s=0.69, s.e.±0.35

[1]All analyses were carried out on separate extracts of 20 g aliquots of fresh pc all taken from the same composite potato preparation.

TABLE 2.—*TGA recovered from potato samples with solanine adde*

Potato TGA, mg[1]	Added solanine, mg	Theoretical total mg	Found, mg	Recover
.78	.44	1.22	1.17	96.3
.40	.84	1.24	1.21	97.6
.78	1.60	2.38	2.24	94.1
.40	4.00	4.40	4.44	100.9

[1]Two separate extracts from different potato sources were used as the base to wl solanine was added.

and titration. No amino nitrogen was found by the titration.

Further evidence that the final solution being titrated is free extraneous amine, is provided by titration values of filtrates from ammo precipitation of the glycoalkaloids. Extracts from several tuber samp were precipitated with NH_4OH. The filtrates from these precipitates w concentrated to dryness, acid hydrolyzed, extracted with benzene a titrated. Very low titers again indicated that the considerable amount amino nitrogen in the extracts was being excluded by the cleanup p cedure. The small titers were attributed to traces of glycoalkaloids precipitatable with NH_4OH.

A number of packages of potatoes were obtained from local mark These were carried through the analytical procedure described here. addition, to provide a comparison with the use of antimony trichlor (2), the glycosides were precipitated by ammonia from an aliquot the original extract. The ammonia precipitates from these samples w permitted to react with antimony trichloride and the optical densities re at 550 nm (3). These values converted to mg TGA/100 g fresh weig of potato were compared with values obtained by the volumetric meth described here. Results are shown in Table 3.

These data indicate that our procedure compares favorably with t antimony trichloride method, and has the advantage of measuring g cosides that would be eliminated by previous methods. The simplicity a safety of this titration method should permit an analytical procedure potato glycoalkaloids to become universally available.

LITERATURE CITED

1. Baerug, R. 1962. Influence of different rates and intensities of light on solani content and cooking quality of potato tubers. Eur. Potato J. 5: 242-251.

TABLE 3.—*Comparison of TGA methods.*[1]

Sample no.	mg TGA/100 g fr. potato	
	By titration	By $SbCl_3$ method
1	8.30	6.25
2	7.02	5.40
3	5.29	4.25
4	7.37	6.00
5	10.40	5.40
6	2.40	2.80
7	4.80	7.20
8	4.00	5.40
9	4.40	4.40
10	2.52	2.80
11	5.20	3.80
12	8.80	5.20

most cases where higher results are obtained with the $SbCl_3$ method, differences e within the experimental error of the methods.

Bite, P., E. Magó-Karácsony, T. Rettegi and A. Uskert. 1970. Solanum Glycosides, V. Determination of Solasodine. Acta Chim. (Budapest) 64: 199-201.

Bretzloff, C. W. 1971. A method for the rapid estimation of glycoalkaloids in potato tubers. Amer. Potato J. 48: 158-162.

Dabbs, D. H. and R. J. Hilton. 1953. Methods of analysis for solanine in tubers of solanum tuberosum. Can. J. Technol. 31: 213-220.

Deahl, K. L., R. J. Young and S. L. Sinden. 1973. A study of the relationship of late blight resistance to glycoalkaloid content of fifteen potato clones. Amer. Potato J. 50: 248-253.

Porter, W. L. 1972. A note on the melting point of α-solanine. Amer. Potato J. 49: 403-406.

Sachse, J. and F. Bachmann. 1969. Über die alkaloidbestimmung in solanum tuberosum L. Z. Lebensm. Unters. Forsch. 141: 262-274.

Schreiber, K. 1957. Naturally occurring plant resistance factors against the Colorado potato beetle (*Leptinotarsa decemlineata*) and their possible mode of action. Züchter 27: 289-299.

Sinden, S. L. and R. E. Webb. 1974. Effect of environment on glycoalkaloid content of six potato varieties at 39 locations. U.S. Dept. Agri. Tech. Bull. 1472.

Talley, E. A., T. J. Fitzpatrick and W. L. Porter. 1970. Chemical composition of potatoes VIII. Effect of variety, location, and year of growth on the conteent of nitrogen compounds. Amer. Potato J. 47: 231-244.

Wang, S. L., C. L. Bedford and N. R. Thompson. 1972. Determination of glycoalkaloids in potatoes (S. tuberosum) with a bisolvent extraction method. Amer. Potato J. 49: 302-308.

Wierzchowski, P. and Z. Wierzchowska. 1961. Colorimetric determination of solanidine and solanine with antimony trichloride. Chem. Anal. (Warsaw) 6: 579-585.